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(54) Title: POLYPEPTIDE DENDRIMERS AS UNIMOLECULAR CARRIERS OF DIAGNOSTIC IMAGING CONTRAST AGENTS, BIOACTIVE SUBSTANCES AND DRUGS

(57) Abstract: The invention describes new polypeptide dendrimers and processes for the synthesis of the same. The polypeptide dendrimers of the invention have a structure which consists of a multifunctional core moiety from which highly branched polypeptide chains, formed by short peptide branching units, extend radially outwards. The outermost branches surround a lower density space with hollows and channels into which bioactive substances employed in diagnosis and therapy can be entrapped or covalently linked. For these properties the said polypeptide dendrimers are particularly useful in a number of areas in biology and medicine as carriers for the delivery of bioactive substances, including drugs, or as carriers of bacterial, viral and parasite antigens, gene-therapy compounds and diagnostic imaging contrast agents.

POLYPEPTIDE DENDRIMERS AS UNIMOLECULAR CARRIERS OF DIAGNOSTIC IMAGING CONTRAST AGENTS, BIOACTIVE SUBSTANCES AND DRUGS

Field of the invention

The present invention relates to polypeptide dendrimers their processes of synthesis and their use as carriers for the delivery of bioactive substances, including drugs, or as carriers of bacterial, viral and parasite antigens, gene-therapy compounds and diagnostic imaging contrast agents.

Prior art

Dendrimers are highly branched polymers in which a number of primary branched chains (monodendrons) irradiating from a multifunctional core moiety originates structures and morphologies quite different from classical hyperbranched and star polymers. (D. A. Tomalia et al., Angew. Chem. Int. Ed. Engl., 1990, 29, 138-175; D. A. Tomalia and H. Dupont Durst, "Topics in Current Chemistry", 1993, 165, 193-313). The structural components of dendrimers namely a) a core moiety, b) interior layers (generations) composed of branching units forming the monodendrons radially attached to the core, and c) an exterior of closely spaced surface groups generate, as the generations increase, spheroidal structures with well-developed internal hollows and channels. The cavities and channels create a microenvironment that can be utilized for the entrapment or the covalent coupling of guest molecules. The stepwise synthesis of polyamidoamine (PAMAM) starburst dendrimers with up to 10 generations and their use as host molecules has been reported in a number of patents and papers. (O. A. Matthews et al., Progr. Polym. Sci., 1997, 23, 1-56). Computer modelling of PAMAM dendrimers has shown how the number and dimensions of cavities depend from a) the number (N_c) of functional groups of the core moiety, b) the number (N_b) of reactive sites of the branching unit and c) the dimensions and rigidity of the branching unit. When N_c=3 or 4 and N_b=2, the PAMAM dendrimer series increases its diameter by approximately 10 Å per generation, evolving from a disk-like shape (generations 0-2) to an oblate spheroid (generations 3,4) to a nearly symmetrical spheroid at generations 5 and higher.

Two conceptually different synthetic approaches for the preparation of high-

generation dendrimer exist: the divergent and the convergent approach. Both approaches are based on a repetition of reaction steps, each repetition accounting for the creation of an additional generation. In the divergent synthesis, the dendrimer is grown stepwise from the core moiety and all reactions are carried out in a single molecule. Since every reaction step occurs incompletely at each of the exponentially growing number of terminals (average selectivity lower than 100%), only limited amounts of defect-free dendrimers are obtained. For instance, an average selectivity of 99.5% per reaction step leads to only 29% yield of pure generation 5 poly(propyleneamine) dendrimer. The purification of dendrimers obtained by the divergent approach can hardly be achieved as they have very similar structures to their by-products. In the convergent approach, the synthesis of dendrimers begins from the periphery and ends at the core by first preparing single monodendrons with the desired number of generations and then joining them to the core moiety. Dendrimers synthesized by this approach can be produced nearly pure since only a constant and low number of reactions are required for any generation-adding step. Dendrimers can be also obtained in fewer steps and higher yields, using pre-branched analogues of both cores (hypercores) and branching units (branched monomers) or, alternatively, following "double exponential" and mixed growth strategies of synthesis.

The structural characteristics of dendrimers namely spheroidal surfaces, internal voids and nanoscopic dimensions have suggested their use as host molecules capable of binding guest molecules either at the interior (dendrimers as endo-receptors) or at the surface (dendrimers as exo-receptors). Various small molecular weight organic molecules have been entrapped into carboxylate-terminated hydrocarbon dendrimers. Acetylsalycilic acid and 2,4 chlorophenoxyacetic acid have been encapsulated within, or near the surface of, PAMAM dendrimers of generation 4, 5 and 6 and the sequestering of 10-20 molecules of dopamine in the channels of PAMAM dendrimers of generation 6 has been studied by use of molecular dynamics calculations. (D.A. Tomalia, Angew. Chem. Int. Ed. Engl., 1990, 29, 138-175). Meijer and colleagues have prepared the "dendritic box" by building up a shell of Boc-phenylalanine on the surface of a poly(propyleneamine) dendrimer of generation 5. (J. F. G. A. Jansen et al.,

Science, 1994, 266, 1226-1229). When the shell is formed in the presence of guest molecules, such as Rose Bengal or tetracyanoquinodimethane, those present in the dendrimer voids are trapped sterically. Liberation of guests is only possible after destruction of the shell i. e. by acidolysis of the Boc groups. The number of guest molecules that can be entrapped is dependent upon the guest size. Only a very limited number of papers dealing with the biocompatibility and pharmacokinetics of dendrimers have appeared. PAMAM dendrimers of generation 3-6 were found to have low toxicity, while the generation 7 dendrimer is toxic in vivo. A high pancreas uptake and an unexplained high urinary output for the seventh generation dendrimer have been also observed. Haemolysis and cytotoxicity have been observed for amine-terminated PAMAMs, but not for their analogues terminating with carboxylate groups. (R. Duncan and N. Malik, Proc. Int. Symp. Control. Relat. Bioact. Mater., 1996, 23, 105-106). Metal dendrimeric chelates have been also studied for diagnostic applications. The Gd (III) chelate of the PAMAM-thiourea-diethylenetriaminepentaacetic acid magnetic resonance imaging contrast agent (Gd(III)-PAMAM-TU-DTPA) remains circulating in blood for longer periods of time than the monomeric chelate, the sixth generation chelate being more effective as contrast agent than chelate conjugates based on polylysine, albumin and dextran supports. By attaching a single monoclonal antibody to a PAMAM dendrimer of generation 2, functionalized at the surface with derivatives of tetraacetic or pentaacetic acid for chelation of ^{90}Y, ^{111}In, ^{212}Bi and Gd(III), the feasibility of monoclonal antibody guided radiotherapy and imaging has been demonstrated. Boronated dendrimer-monoclonal antibody conjugates have been used successfully as protein probes in electron spectroscopic imaging. The transfection of antisense oligonucleotides into a variety of cell lines has been carried out in vitro using PAMAM dendrimers. Furthermore, polypeptide monodendrons of generation 2 and 3, composed of lysyl residues (MAP, multiple antigen peptides), have been prepared as branched multivalent scaffolds for peptide conjugation and used as immunogens and immunodiagnostics. (J.P. Tam, J. Immunol. Methods, 1996, 196, 17-32). The author did not however mention the possibility to prepare polypeptide dendrimers of globular shape resembling high generation spheroidal poly(amidoamines) for the encapsulation of guest molecules

in their internal cavities.

The preliminary observations on the in vitro and in vivo properties of PAMAM dendrimers as well as the harsh conditions that are needed to release guest molecules from the dendritic boxes, indicate that both microcontainers are not suitable as carriers for bioactive substances and drug delivery. Besides favourable pharmacokinetic properties, such carriers should have:

1) biological stealthiness (biocompatibility).

2) limited and controlled stability towards enzymes. Enzymatic processing is necessary not only to avoid the chronic toxicity due to non-specific accumulation in the body, but also to obtain the controlled release of guest molecules by gradual hydrolysis of the dendrimer structure.

3) high carrying capacity. The internal voids of PAMAMs are not big enough to encapsulate either a large number of low molecular weight molecules or a reasonable number of macromolecular guests like, for instance, insulin.

4) controlled dimensions, preferably in the 10-100 nm range, to avoid rapid urinary clearance and RES (reticuloendothelial system) uptake.

Summary of the invention

The applicant has now surprisingly found that dendrimers with a polypeptide backbone can have the properties above mentioned and comply with the following aims of the present invention. A first aim of the present invention is that of providing water soluble polypeptide carriers with dendrimeric structures, spheroidal shapes and precisely defined dimensions (unimolecular dendrimeric carriers), with channels and cavities that can host bioactive substances and drug molecules with molecular weights up to 5-7 kDa. A second aim of the present invention is that of providing polypeptide dendrimeric carriers whose gradual demolition in vivo, in blood or at the target cellular sites, occurs both by enzymatic hydrolysis (which can be controlled and modulated by insertion of D aminoacid residues into the backbone) and by UV irradiation if the carriers contain photolabile bonds. A third aim of the present invention is that of providing loaded polypeptide dendrimeric carriers whose dimensions and surfaces are tailored to avoid RES uptake as well as rapid urinary clearance. An additional aim of the present invention is the synthesis of polypeptide dendrimeric carriers with antigen moieties

(peptides, oligonucleotides, saccharides and oligosaccharides deriving from relevant pathogenic agents) covalently linked to their surface reactive groups. A further aim of the present invention is the derivatisation of the surface of the polypeptide dendrimeric carriers with biological receptor ligands such as folic acid, sialic acid, mannose, fat acids, vitamins, hormons, oligonucleotides, monoclonal antibodies, short peptides, proteins and oligonucleotides for cell targeting.

Then, the object of the present invention are polypeptide dendrimers having:

i. a multifunctional core moiety;

ii. an exterior of closely spaced groups constituting the terminals of branched polypeptide chains (monodendrons) radially attached to the core that, in turn, form

iii. interior layers (generations) of short peptide branching units (propagators) with characteristic hollows and channels, where each propagator contains a trifunctional aminoacid whose asymmetric carbon (the propagator branching point) is connected to two equal-length arms bearing identical terminal reactive groups and to a third arm (the propagator stem) bearing an activatable functional group, represented by formula (I):

$$K(-L)_p-M \qquad (I)$$

wherein

K is a multifunctional core moiety,

L is a polypeptide monodendron,

p is the number of polypeptide monodendrons irradiating from the core moiety and

M represents the outermost ramifications of the dendrimer.

Further objects of the present invention are the processes for the synthesis of said polypeptide dendrimers and the use in biology and medicine of the same as carriers for the delivery of bioactive substances, including drugs, or as carriers of bacterial, viral and parasite antigens and gene-therapy compounds and diagnostic imaging contrast agents.

Detailed description of the invention

The polypeptide dendrimers, the processes for their synthesis and the use as unimolecular carriers, according to the present invention, will be better illustrated in the following description.

The polypeptide dendrimers of this invention consist of highly branched

polypeptide chains or monodendrons, deriving from repeated condensations of short peptide branching units or propagators, that irradiate outward from a multifunctional core moiety, having an exterior of closely spaced groups constituting the terminals of the monodendrons, and interior layers or generations of propagators with characteristic hollows and channels where each propagator contains a trifunctional aminoacid whose asymmetric carbon (the propagator branching point) is connected to two equal-length arms bearing identical terminal reactive groups and to a third arm (the propagator stem) bearing an activatable functional group. The polypeptide dendrimers are represented by formula (I):

$$K(-L)_p\text{-}M \qquad (I)$$

wherein: K is the multifunctional core moiety and K can be represented by the formulae:

(II) $X\text{-}(CH_2)_n\text{-}X^1$,

wherein $X=X^1$ or $X\neq X^1$, and X, X^1 are NH or CO or S; or

(III) $Y[\text{-}(CH2)_n\text{-}Z]_i$,

wherein Y=C or Y=N; Z is NH or S or Cl or Br or I or a maleimide residue, n=1-6 and i=3,4;

or (IV) $X\text{-}CH(R)\text{-}CO[\text{-}NH\text{-}CH(R)\text{-}CO]_n\text{-}NH\text{-}CH(R)\text{-}COOR^1$,

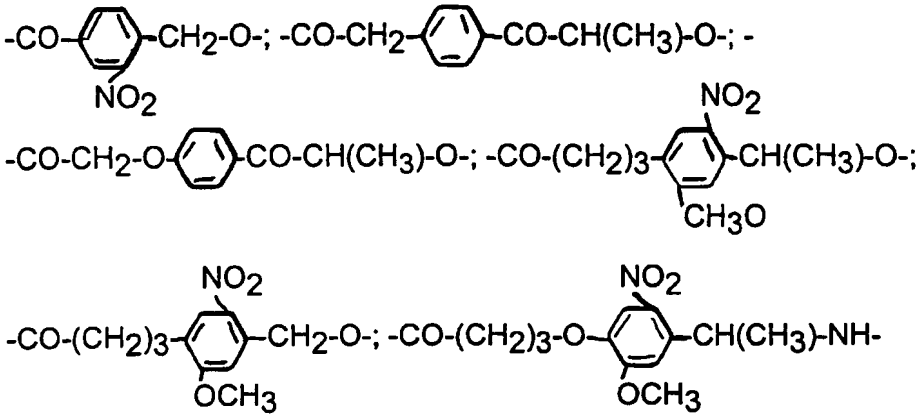
wherein R is $(CH_2)_m\text{-}X^1$, m=1-5, R^1 is methyl or ethyl or butyl or isopropyl, $X=X^1$ or $X\neq X^1$, and X, X^1 are NH or CO or S and n=1-6;

L is the single monodendron whose propagators can be represented by the formulae: (V) $\text{-}CO\text{-}CH(R^2)\text{-}(CH2)_n\text{-}NR^3\text{-}$

wherein R^2=H or the side-chain of a natural or synthetic aminoacid, and their derivatives; R^3=H or a linear hydrocarbon radical optionally substituted with OH or SH or Cl or Br; $R^2\text{-}CH(CH2)_n\text{-}NR^3$ is a 5 or 6 atoms ring, and n=0-6; and

(VI) $\text{-}CO\text{-}CH(R^2)\text{-}CO\text{-}N(R^3)\text{-}(CH2)_m\text{-}N(R^3)$

wherein R^2 and R^3 have the meaning seen above and m=1-6; or L is the single monodendron whose propagators can be represented by one of the residues: $\text{-}CO\text{-}CH_2\text{-}NH\text{-}NH$; $\text{-}CO\text{-}CH(R^2)\text{-}O\text{-}$; $\text{-}CO\text{-}CH_2\text{-}O\text{-}N{=}CH\text{-}CO\text{-}$; $\text{-}CO\text{-}CH(R^2)\text{-}(CH2)_n\text{-}S\text{-}CH_2\text{-}CO\text{-}W$; $\text{-}CO\text{-}NH\text{-}CH(CH_2\text{-}SH)\text{-}CO\text{-}W$; $\text{-}CO\text{-}N(HO\text{-}CH_2\text{-}CH\text{-}T\text{-}CH\text{-}Q)\text{-}CH\text{-}CO\text{-}W$;

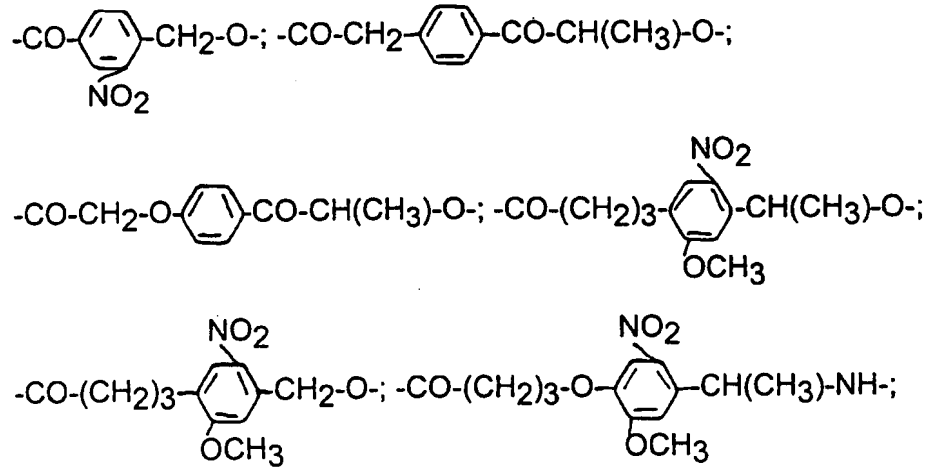


wherein W=-N(R³)-(CH$_2$)$_m$-NR³, Q=H, -CH$_3$; T is O or S while R², R³ and m have the meaning seen before and p=1-4;

M is the residue represented by formula (VII):

-A$_q$-B(A$_r$)-C-A$_r$[A$_q$-B(A$_r$)-C-A$_r$[A$_q$-B(A$_r$-D)-C-A$_r$-D]$_2$]$_2$ (VII)

wherein A=-CO-CH(R²)-(CH$_2$)$_n$-NR³; R³ and n have the meaning seen before; q=1-6; r=1-4 and R², in addition to the meaning seen before, is a natural or synthetic trifunctional aminoacid; B is -CO-CH[-(CH$_2$)$_n$-X¹]-X, with X=X¹ or X≠X¹; X and X¹ are NH or CO or S; n=1-5; C=A or -CO(CH$_2$)$_n$-NH; -(CH$_2$)$_n$-S with n=1-6; or C is one of the residues:



D is a residue represented by formulae (VIII)-(XI):

-A$_q$-B(A$_r$-E)-C-A$_q$-E (VIII)

-A$_q$-B(A$_r$)-C-A$_q$[A$_q$-B(A$_r$-E)-C-A$_q$-E]$_2$ (IX)

-A$_q$-B(A$_r$)-C-A$_q$[A$_q$-B(A$_r$)-C-A$_q$-[A$_q$-B(A$_r$-E)-C-A$_q$-E]$_2$]$_2$ (X)

-A$_q$-B(A$_r$)-C-A$_q$[A$_q$-B(A$_r$)-C-A$_q$-[A$_q$-B(A$_r$)-C-A$_q$[A$_q$-B(A$_r$-E)-C-A$_q$-E]$_2$]$_2$]$_2$ (XI)

wherein A, B, C, q ed r have the meaning seen before, and E is represented by formulae (XII) and (XIII):

$-A_q-B(A_r-P)-C-A_q-P^1$ (XII)

$-A_q-B(A_r)-C-A_q[-A_q-B(A_r-P)-C-A_q-P^1]_2$ (XIII)

wherein A, B, C, q and r have the meaning seen before; $P=P^1$ or $P\neq P^1$; P and P^1 being H or a linear hydrocarbon radical optionally substituted with one or more linear or branched alkyl groups, acyl, aminoacid, peptide, nucleotide, oligonucleotide, saccharide, oligosaccharide, protein, monoclonal antibody, polyethylenglycol containing 10-400 $-CH_2-CH_2-O-$ repeats, lipid, enzyme, metal ligand. The terms aminoacid, peptide, nucleotide, oligonucleotide, saccharide, oligosaccharide, protein comprise either natural or synthetic analogues and derivatives.

A characteristic feature of the polypeptide dendrimers of the present invention is the limited stability of their backbone to plasma and cellular enzymes and, more important, the possibility of programming the stability towards enzymes in vivo by replacing L with D aminoacids. This property distinguishes the polypeptide dendrimers from PAMAM, polypropylamine, hydrocarbon, polyether, polythioether and silicon-based dendrimers that, being all stable to enzymatic hydrolysis, may accumulate non-specifically in the body creating toxicity problems. By regulating both the polypeptide dendrimer dimensions (from 10 to 100 nm, to avoid rapid urinary excretion and uptake by the RES system) and the liability of the dendrimer backbone, it is feasible to balance the retention and the excretion of the polypeptide dendrimeric carriers in the body. In addition to enzyme hydrolysis, the demolition of polypeptide dendrimers with release of guest molecules can be obtained by ultraviolet irradiation of selected bonds when a limited number of photolabile residues are inserted in the backbone instead of aminoacid residues. As a result, the release of bioactive guest molecules or drugs can be triggered at the site of therapeutic utility with generation of fewer systemic side-effects.

The applicant has surprisingly found that polypeptide dendrimers can be prepared, in accordance with the present invention, by condensing to a core moiety with 2, 3 or 4 identical functional group, two, three or four polypeptide monodendrons, previously prepared by stepwise synthesis, using short three-branched peptide

propagators as building blocks. Alternatively, low-generations monodendrons can be condensed to a preformed dendrimer (expanded core) to obtain the final dendrimer. The polypeptide dendrimers of the present invention not only encapsulate guest molecules of a wide range of molecular weights but, surprisingly, show also an extraordinary solubility in water even when surface polar groups such as NH_2, OH, and COOH are masked by hydrophobic moieties. Below are reported methods and examples that demonstrate: 1) the feasibility of the chemical synthesis of polypeptide dendrimers; 2) the possibility of entrapment and encapsulation of guest molecules into the dendrimeric carriers; 3) the release of guest molecules by enzymatic hydrolysis and by ultraviolet irradiation in vitro and in vivo; and 4) the non-immunogenicity and adjuvanticity of polypeptide dendrimers in mice. Numerous embodiments and other features of the present invention will become better understood with reference to the following descriptions.

General methods of synthesis

According to the present invention, a first general process for the preparation of unimolecular polypeptide dendrimers consists in: 1) the synthesis of core moieties with at least two functional groups; 2) the divergent synthesis of single polypeptide monodendrons; 3) the covalent conjugation of the polypeptide monodendrons to the core moieties. A second general process for the preparation of polypeptide dendrimers consists in: 1) the synthesis of core moieties with at least two functional groups; 2) the condensation of monodendrons of generation 1-3, protected at their termini with removable groups, to the core moieties; 3) the removal of protecting groups from the low generation dendrimers obtained in step 2 followed by the reiterated condensation of protected monodendrons to reach the target high generation dendrimers; and 4) the removal of protecting groups from the final dendrimer followed by surface modification, when necessary. Protecting groups, condensing and deblocking agents, solvents and reaction times are selected considering not only the structure of both core moieties and propagators, but also the chemical and structural properties of guest molecules.

According to the general formula (I) of the present invention and following the two general processes above outlined it is possible, for example, to synthesize

structurally simple polypeptide dendrimers characterized by a bifunctional core such as ethylenediamine to which single monodendrons of generation from 3 to 7 are covalently linked namely $_2$(2(2(H-Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly-HN-CH_2-CH_2-NH-Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly-H)$_2$)$_2$)$_2$ and $_2$(2(2(2(2(2(H-Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly-HN-CH_2-CH_2-NH-Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly-H)$_2$)$_2$)$_2$)$_2$)$_2$)$_2$)$_2$.

The objective of entrapping into polypeptide dendrimers molecules with molecular weights above 1,000 Da is obtained in two steps: 1) assembly of polypeptide monodendrons on solid supports (Solid-Phase Peptide Synthesis, SPPS), using short peptide derivatives as building blocks (divergent strategy) and 2) condensation, in aqueous phase and in the presence of guest molecules, of the polypeptide monodendrons to the core moiety by "chemical ligation" methods as currently applied for the synthesis of proteins (P.Lloyd-Williams, F. Albericio and E. Giralt, "Chemical Approaches to the Synthesis of Peptides and Proteins", 1997, CRC Press, Boca Raton, 175-200).

The objective of encapsulating into the polypeptide dendrimer molecules with molecular weight below 1,000 Da is obtained both by the above strategy of trapping guest molecules during dendrimer synthesis and also by first preparing "void carriers" that are subsequently filled up by diffusion of small guest molecules in their cavities. The objective of preparing polypeptide dendrimers with photolabile bonds is obtained following the above methods and using monodendrons with one or more aminoacid residues of the backbone replaced by photolabile moieties. The objective of preparing polypeptide carriers with guest molecules covalently linked at their interior is obtained by 1) preliminary entrapment of guest molecules into the dendrimer cavities by diffusion and 2) covalent coupling to the reactive groups of the dendrimer carrier. Finally, the objective of conjugating biologically active molecules to the surface of polypeptide dendrimers for receptor targeting is obtained by covalent condensation of a reactive group of the bioactive molecule that is not critically important for receptor recognition.

Numerous embodiments and other features of the present invention will become better understood with reference to the following descriptions. The examples reported below are not intended to limit the present invention and further modifications deriving from the natural advancement of the synthetic and dendrimer loading protocols are within the spirit and the scope of the present invention.

The HPLC analysis was carried out with a Bruker LC21-C apparatus equipped with the UV Bruker LC313 detector, using Pico Tag Waters columns and acetonitrile-water buffers A) 10% (v/v) acetonitrile in 0.1% TFA water and B) 60% (v/v) acetonitrile in 0.1% TFA water; gradient (I) from 0 to 100% B in 25 min and (II) from 50 to 100% B in 25 min; flow, 1 ml/min, 220 nm detection. Peptide purification by preparative HPLC was carried out with the Waters Delta Prep 3000 apparatus on a Delta Pack C18-300Å (30 mm ^{X} 30 cm, 15 μ) column, with the same eluants and conditions. Flow, 30 ml/min, 220 nm detection. Thin layer chromatography was carried out on F 254 silica gel plates (Merck), using as eluant 1-buthanol/ acetic acid/water (3:1:1 v/v/v). 1% ninhydrin in ethanol and Cl_2-Iodine were used as detecting reagents. ^1H NMR measurements were made with the 200 MHz FT Bruker apparatus. Molecular weights were confirmed by mass spectrometry on a Voyager-DE apparatus (PerSeptive Biosystems, MA, USA).

EXAMPLE 1

This example describes the synthesis of a generation 4 dendrimer by condensation in liquid phase of a generation 4 monodendron derivative assembled on a solid-matrix, to a triamine core.

1. Synthesis of $N[CH_2\text{-}CH_2\text{-}NH\text{-}CO\text{-}CH(CH_2\text{-}phenyl)\text{-}NH_2]_3 \cdot 4HCl$

1.91 g of Boc-Phe-OH (7.2 mmole), 150 μl of $N(CH_2\text{-}CH_2\text{-}NH_2)_3$ (2.0 mmole), 1.43 g of WSC·HCl (7.5 mmole), 1.15 g of HOBt (7.5 mmole) and 560 μl of triethylamine (4.0 mmole) were dissolved in 10 ml of anhydrous DMF at 0 °C and kept under agitation for 24 h at room temperature. After evaporation of DMF, the solid was dissolved in 100 ml of ethyl acetate and extracted with 5% $NaHCO_3$ (3^X20 ml) and brine (3^X20 ml). The organic solution was acidified, the solvent

evaporated and the resulting product, dissolved in 70 ml of ethyl acetate, further treated with 4N HCl at 0°C. The mixture was left under agitation at room temperature for 30 min. The residue obtained after evaporation of the solvent was dissolved in 20 ml of methanol and precipitated with ethyl ether-petroleum ether (1/1, v/v). The solid obtained after filtration was washed repeatedly with ethyl ether-petroleum ether (1/1, v/v). M.p.: 167 °C; $[\alpha]_D^{22}$ -1.8 (c1, DMF); R.f.: 0.5; HPLC: 8,97 min; gradient (I); MS: 589 Da, 611 Da and 627 Da, for M-H^+, M-Na^+, M-K^+ , respectively.

2. Synthesis of Fmoc-Gly-Orn(Fmoc)-Gly-Gly-OH

A solution of 14.9 g of Boc-Orn(Fmoc)-OSu (27 mmole) in 30 ml of DMF was added under agitation at 0°C to a solution of 3. 92 g of H-Gly-Gly-OH (29.7 mmole) in 45 ml of 5% $NaHCO_3$ and 100 ml of DMF. After 1 h at 0°C the reaction is continued overnight at room temperature. After DMF evaporation, the residue was dissolved in 150 ml of 10% citric acid and the product extracted with 200 ml of ethyl acetate. The solution was then washed with brine, dried over Na_2SO_4, filtered and concentrated to a final volume of 50 ml by elimination of the solvent. The product was recovered by precipitation with 150 ml of ethyl ether containing 2 ml of methanol. Yield, 13.9 g. M.p.: 125-128°C; R.f.: 0.7 in 1-butanol/ acetic acid /water (3:1:1, v/v/v); HPLC: 19.25 min; gradient (I).

13.9 g of Boc-Orn(Fmoc)-Gly-Gly-OH were dissolved in 20 ml of TFA and kept for 1 h at room temperature. After TFA evaporation, the residue was triturated with ethyl ether and dried. The salt obtained (14.5 g of TFA·H-Orn(Fmoc)-Gly-Gly-OH, 24.8 mmole), was dissolved at 0 °C in 50 ml of 5% $NaHCO_3$ and 150 ml of DMF and left to react with 8.78 g of Fmoc-Gly-OSu (22.3 mmole) for 1 h at 0 °C and overnight at room temperature. After DMF evaporation, the residue was dissolved in 10% citric acid, filtered and washed several times with water. The crude product was crystallised from ethyl acetate. Yield: 14 g; M.p.: 208-210 °C; R.f.: 0.63; HPLC, 23.68 min; gradient (I); $[\alpha]_D^{22}$-20 (c1, DMF).

NMR (DMSO) δ ppm: 1.32-1.8, m 4H; 2.92-3.06, m 2H; 3.65-3.79, m 6H; 4.18-4.36, m 7H; 7.31-7.9, m 18H; 7.98, d 1H; 8.1, t 1H; 8.25, t 1H; 12.5, bs 1H. MS:

748 Da.

3. Synthesis of 2[2[2[Ac-Gly-Orn(Ac)-Gly-Gly]Gly-Orn-Gly-Gly]Gly-Orn-Gly-Gly)]Gly-Orn-Gly-Gly-OH.

The synthesis was carried out on a Milligen 9050 machine, using a 0.5 cm (I.D.) column, loaded with 0.5 g of Fmoc-Gly-PEG-PS (Millipore) resin. Loading: 0.18 mmole/g.

1st cycle: a) deprotection: 20% piperidine in DMF, 4 min, flow: 8.1 ml/min; b) whashing: DMF, 10 min, flow: 4.0 ml/min; c) coupling: 134 mg of Fmoc-Gly-Orn(Fmoc)-Gly-Gly-OH, 68 mg of HBTU and 28 mg of HOBt were dissolved manually in 0.6 ml of 0.6M N-methylmorpholine (NMM) in DMF and 0.4 ml of DMF and then loaded into the column (automatic protocol). Recycle: 5 h, flow: 8.1 ml/min; d) washing: DMF, 15 min, flow: 4.0 ml/min.

2nd cycle: 268 mg of Fmoc-Gly-Orn(Fmoc)-Gly-Gly-OH, 136 mg of HBTU and 56 mg of HOBt dissolved in 1.2 ml of 0.6 M NMM in DMF and 0.3 ml of DMF were employed for coupling. A small sample of resin was extracted from the column, treated with 20% piperidine in DMF, carefully dried and treated again with TFA/water (95/5, v/v) for 1h at room temperature. A single HPLC peak a 2.8 min, gradient (I), was observed.

3rd cycle: Two couplings were performed. In the first coupling, 400 mg of Fmoc-Gly-Orn(Fmoc)-Gly-Gly-OH, 208 mg of HBTU and 80 mg of HOBt dissolved in 1.8 ml of 0.6 M NMM in DMF and 0.2 ml of DMF were employed. Three consecutive washings with DMF (20 min, flow: 4.0 ml/min), DCM (10 min, flow: 9.0 ml/min) and DMF (5 min, flow 4.0 ml/min) were carried out. In the second coupling, 200 mg of Fmoc-Gly-Orn(Fmoc)-Gly-Gly-OH, 104 mg of HBTU and 40 mg of HOBt dissolved in 0.9 ml of 0.6 NMM in DMF and 0.1 ml of DMF were employed. Recycle: 3h; flow: 8.1 ml/min; three washings with DMF and DCM as before. A small sample of resin, extracted from the column and analysed as before, gave a single HPLC peak at 6.3 min with gradient (II).

4th cycle: Two couplings were performed. 800 mg of Fmoc-Gly-Orn(Fmoc)-Gly-Gly-OH, 416 mg of HBTU, 160 mg of HOBt dissolved in 3.6 ml of 0.6 M NMM in DMF and 0.4 ml of DMF were employed for the first coupling. Recycle: 3;5 h; flow: 8.1 ml/min; three washings with DMF, DCM and DMF. In the second coupling, 400

mg of Fmoc-Gly-Orn(Fmoc)-Gly-Gly-OH, 208 mg of HBTU and 80 mg of HOBt dissolved in 1.8 ml of 0.6 M NMM in DMF were employed. Recycle: 5 h; flow: 8.1 ml/min. The resin was washed and analysed as before. A single, broader HPLC peak was observed at 8.1 min; gradient (II). The resin was then treated with 20% piperidine in DMF for 10 min at a flow of 8.1 ml/min, washed for 15 min with DMF at a flow of 4.0 ml/min. and acetylated with 1 M acetic anhydride and 1 M NMM in DMF for 1 h, flow: 8.1 ml/min. Finally, the resin was extracted from the column, washed with DMF, methanol, DCM and ethyl ether and dried under vacuum overnight. The peptide monodendron was obtained by suspending the resin in 15 ml of TFA/water (95/5, v/v) for 1 h at room temperature under stirring. After filtration, the resin was washed with 1 ml of TFA and the combined filtrates, after partial evaporation of TFA, were added to cold ethyl ether to precipitate the polypeptide. The mixture was kept at -20 °C for about 3 h. After filtration, the white product was dissolved in water and lyophilized three times. Yield: 420 mg. A dominating, broad HPLC peak was observed at 8.1 min, gradient (I), together with two very small peaks corresponding to products of the second and third cycle. The product has been purified by Size Exclusion Chromatography (SEC) on Sephadex G-50, using 50% acetic acid as eluant. The fractions containing the target peptide were lyophilized twice after dilution with water. Yield: 350 mg. MS: 5,021 Da (Theor. 5.022 Da).

4) Synthesis of N{CH_2-CH_2-NH-CO-CH(CH_2-phenyl)-NH-Gly-Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-GlyOrn(Ac)GlyAc]$_2$]$_2$]$_2$}$_3$

7.33 mg of N[CH_2-CH_2-NH-CO-CH(CH_2-phenyl)-NH_2]$_3$·4HCl (0.01 mmole), 200.1 mg of the monodendron prepared as reported in 3) (0,04 mmole), 9.6 mg of WSC·HCl (0.5 mmole), 7.7 mg of HOBt (0.5 mmole) and 5.6 µl of triethylamine (TEA) (0.04 mmole) were dissolved in 15 ml of DMF, treated with TEA to reach an apparent basic pH, and left to react for 48 h at room temperature under stirring. After DMF evaporation, the residue was dissolved in 10 ml of methylethylketone and the solution extracted with 5% $NaHCO_3$ (3X10 ml) and brine (3X10 ml), acidified with 0.1 M HCl and dried over Na_2SO_4. The solid recovered after

evaporation of the solvent was washed four times with ethyl ether, dried under vacuum, dissolved again in 50% acetic acid and purified by SEC on Sephadex G-50 as reported before. Yield: 161 mg; MS: 15,605 Da (Theor: 15,602 Da). The product has been characterised further by SEC HPLC using a 75HR10/30 Pharmacia Superdex column (stationary phase: cross-linked agarose-dextran, 13 μm), using 50 mM NaH_2PO_4 and 100 mM Na_2SO_4 pH 6,5 as eluants; flow: 0.5 ml/min; detection, 220 nm. A single broad peak was observed at 18 min. Ribonuclease (MW=13,400 Da), Bovine Serum Albumin (BSA) monomer (MW=66,000 Da) and dimer (MW=112,000 Da) show peaks at 25, 20 and 18 min, respectively. These results indicate that the acetylated generation 4 dendrimer aggregates in the buffer used for SEC HPLC.

EXAMPLE 2

This example describes a three step synthesis of a generation 4 dendrimer prepared entirely in liquid-phase. In the first step, a generation 2 monodendron with NH_2 terminals protected by an acid labile group is condensed on a triamine core to obtain a generation 2 dendrimer. In the second step, after acidolysis, the monodendron is again condensed to the free NH_2 terminals of the generation 2 dendrimer to obtain a generation 4 dendrimer. In the third step, after removal of the protecting groups, the dendrimer NH_2 terminals are acetylated.

1) Synthesis of Z-Orn(Boc)-Gly-Gly-OCH_3

10.44 g of Z-Orn(Boc)-OH (28.5 mmole), 5.75 g of WSC·HCl (30 mmole), 4.59 g of HOBt (30 mmole), 5.47 g of HCl·H-Gly-Gly-OCH_3 (30 mmole), and 5.6 ml of TEA (40 mmole) were dissolved in 90 ml of DMF, treated with TEA until basic pH and then left to react for 12 h at room temperature under stirring. After DMF evaporation, the residue is dissolved in 300 ml of ethyl acetate and washed with 0.1 M HCl/brine 1/2 (3 x 40 ml), 5% $NaHCO_3$/brine 2/1 (5 x 40 ml) and again brine (30 ml). The solution is then acidified with 0.1 M HCl and dried over Na_2SO_4. The solvent is then almost completely evaporated and the target product recovered by slow crystallization from ethylether/petroleum ether 1/1 v/v. Yield: 13.7 g. A single HPLC peak was observed at 18.2 min; gradient (I).

2) Synthesis of Boc-Gly-Orn(Boc)-Gly-Gly-OH

13 g of Z-Orn(Boc)-Gly-Gly-OCH_3 were dissolved in 170 ml of methanol and treated with 750 mg of 10% C/Pd. Hydrogenation is continued for 2 h at room temperature. After elimination of the solid by filtration the resulting solution is concentrated and the product slowly crystallized from ethyl ether petroleum ether 1/1. Yield: 9.7 g.

8,83 g of H-Orn(Boc)-Gly-Gly-OCH_3 (24.5 mmole), 6.26 g of Boc-Gly-OSu (23 mmole) were dissolved in 30 ml of DMF. 10 mmole of TEA were added to the solution after 7 h at 0°C. The reaction was continued for 24 h at room temperature. Following DMF evaporation and addition of 300 ml of ethyl acetate, the organic solution was extracted 1 M HCl/brine 1/2 (3 x 30 ml), 5% $NaHCO_3$/brine 1/1 (3 x 30 ml) and brine (2 x 30 ml). After acification with 1 M HCl, and solvent evaporation, the product was isolated by crystallization from ethyl ether. Yield: 11.8 g. A single HPLC peak was observed at 15.5 min; gradient (I).

5,18 g of Boc-Gly-Orn(Boc)-Gly-Gly-OCH_3 (10 mmole) were reacted with 1 M NaOH in methanol (50 ml) for 15 h at room temperature. After alcohol evaporation, the residue was dissolved in 200 ml of ethyl acetate and extracted with 30 ml of 1 M HCl saturated with NaCl and brine (2 x 20 ml). After acidification and solvent elimination, the product was isolated by crystallization from ethyl ether/petroleum ether 1/1 v/v. Yield: quantitative. A single HPLC peak was observed at 14.48 min; gradient (I). MS: 527 Da, 543 Da and 565 Da for M-Na^+, M-K^+ and M-K^+-Na^+ (Theor: 504 Da).

3) Synthesis of [Boc-Gly-Orn(Boc)-Gly-Gly]$_2$-Gly-Orn-Gly-Gly-OH

4,03 g of Boc-Gly-Orn(Boc)-Gly-Gly-OH (8.0 mmole), 1.48 g of HCl·H-Gly-Orn(HCl)-Gly-Gly-OCH_3 (3.8 mmole), 1.69 g of WSC·HCl (8.8 mmole), 1.35 g of HOBt (8.8 mmole) and 1.12 ml of TEA (8.0 mmole) were dissolved in 30 ml of DMF at 0°C. The reaction was kept for 15 h at room temperature under stirring. After DMF evaporation, the residue was dissolved in 200 ml of methylethylketone. The solution was extracted with 1 M HCl/brine 1/1 (4 x 20 ml), 5% $NaHCO_3$ (3 x

20 ml) and brine (3 x 20 ml). After acidification with 1 M HCl, the solvent was evaporated and the product crystallized from ethyl acetate/ethyl ether ¼ v/v. Yield: ca. 4 g of methyl ester were isolated after three further washings with ethyl ether. 3.97 g were dissolved in 50 ml of warm methanol, the solution left to equilibrate at room temperature and then treated with 4 ml of 1 M NaOH for 16 h. After evaporation of the solvent, the residue was dissolved in 200 ml of methylethylketone and 10 ml of 1 M HCl and 20 ml of brine were added to the solution. The solution was carefully extracted and neutralized with brine (3 x 30 ml). The organic layer was then dried on Na_2SO_4, filtered and the solvent evaporated. The crude product was crystallized from ethyl acetate and the solid obtained washed three times with ethyl ether. Yield: 3.7 g. A single HPLC peak appeared at 19.14 min; gradient (I). MS: 1,298 Da and 1,314 Da for M-Na^+ and M-K^+ (Theor.: 1,275 Da).

4) Synthesis of N{CH_2-CH_2-NH-CO-CH(CH_2-phenyl)-NH-Gly-Gly-Orn-Gly[Gly-Gly-Orn(Boc)-Gly-Boc]$_2$}$_3$

510 mg of [Boc-Gly-Orn(Boc)-Gly-Gly]$_2$-Gly-Orn-Gly-Gly-OH (0.4 mmole), 73.3 mg of N[CH_2-CH_2-NH-CO-CH(CH_2-phenyl)-NH_2]$_3$·HCl (0.1 mmole), 96.0 mg of WSC·HCl (0.5 mmole), 77.0 mg of HOBt (0.5 mmole) and 56 µl of TEA were dissolved in 20 ml of DMF at room temperature. TEA was added to a basic pH and the mixture left to react for 48 h under stirring. After DMF evaporation, the residue is dissolved in 100 ml of methylethylketone and the solution extracted with 0.5% $NaHCO_3$ (3 x 20 ml) and brine (3 x 20 ml). Following acidification with 1 M HCl, the organic solution is dried over Na_2SO_4, filtered and evaporated to obtain a white powdery solid which was repeatedly washed with ethyl ether. Yield: 450 mg. A single HPLC peak was observed at 22.69 min; gradient (I). MS: 4,359 Da and 4,381 Da for M-H^+ and M-Na^+ (Theor.: 4,355 Da).

5) Synthesis of N{CH_2-CH_2-NH-CO-CH(CH_2-phenyl)-NH-Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn(Ac)-Gly-Ac]$_2$]$_2$]$_2$}$_3$

436 mg di N{CH_2-CH_2-NH-CO-CH(CH_2-phenyl)-NH-Gly-Gly-Orn-Gly[Gly-Gly-

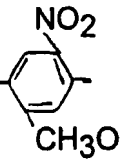
Orn(Boc)-Gly-Boc]$_2$}$_3$ (0.1 mmole) were dissolved in 2 ml of warm DMSO. 15 ml of 4 M HCl in dioxane were then added to the solution left to equilibrate at room temperature and the reaction kept for 1 h under stirring. The salt obtained was triturated and isolated by centrifugation at 2,000 rpm. After two washings with ethyl acetate, the hygroscopic product was dried under vacuum over P_2O_5.

363 mg of salt, (0.1 mmole) were dissolved in 2 ml of water, neutralized with 0.1 M NaOH and added to a 5 ml DMF solution containing 1.53 g of [Boc-Gly-Orn(Boc)-Gly-Gly]$_2$-Gly-Orn-Gly-Gly-OH (1.2 mmole), 250 mg of WSC·HCl (1.3 mmole), 200 mg of HOBt (1.3 mmole) and 210 µl of TEA (1.5 mmole). The solution was left to react for 48 h at room temperature under stirring. After DMF evaporation, the solid was dissolved in 50 ml of methylethylketone and the solution extracted with 5% $NaHCO_3$ (3 x 20 ml) and brine (3 x 20 ml). After acidification with 1 M HCl and drying over Na_2SO_4, the organic layer was filtered and evaporated to obtain a solid residue which was, in turn, repeatedly washed with ethyl ether. The solid, dried under vacuum, was again dissolved in 20 ml of TFA/water 98/2 v/v and left to react for 2 h under agitation. The residue obtained after solvent elimination was repeatedly washed with ethyl ether and dried under vacuum.

800 mg of trifluoroacetate salt (3.6 mmole) were dissolved in 10 ml of DMF/water 1/1 v/v with 905 mg of p-nitrophenylacetate (5.0 mmole) and 700 µl of TEA (5 mmole). The solution was left to react for 50 h. After evaporation of the solvent, the residue was repeatedly washed with ethyl ether and dried under vacuum. Yield: 1.1 g. The crude was purified by SEC on Sephadex G-50, with 50% acetic acid as eluant. The fractions containing the target product were pooled and lyophilized. MS: 15,439 Da (Theor.: 15,431 Da). The MW of the dendrimer has been also determined by SEC HPLC, using a 75HR10/30 Pharmacia Superdex column, as described in Example 1). R.t.: 18 min. The dendrimer is then identical to that prepared following the strategy of Example 1).

EXAMPLE 3

This example illustrates the synthesis of a generation 7 dendrimer containing 4-[4-(1-(amino)ethyl)-2-methoxy-5-nitrophenoxy]butanoic acid photocleavable residues.

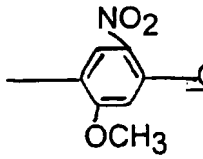
1) Synthesis of HOOC-CH(CH_2-SH)-NH-CO(CH_2)$_3$-O- 

CH(CH_3)-NH-Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn(Ac)-Gly-Ac]$_2$]$_2$]$_2$]$_2$]$_2$]$_2$]$_2$

The synthesis was carried out on a Milligen 9050 apparatus, using a 0.5 cm (I.D.) column, loaded with 0.1 g of Fmoc-Cys(Trt)-PEG-PS (Millipore) resin. Loading: 0.16 mmole/g.

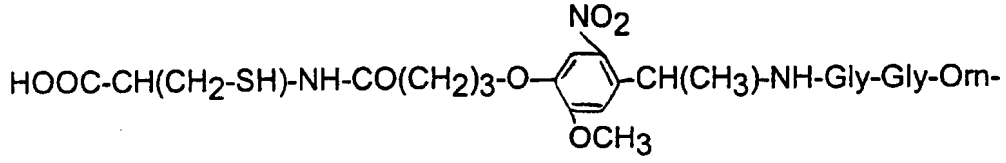
In the first cycle of the chain assembly, 4-[4-(1-Fmoc-aminoethyl)-2-methoxy-5-nitrophenoxy]butanoic acid was condensed to the cysteine(Trt) residue on the resin after Fmoc removal with 20% piperidine in DMF. All subsequent cycles for the synthesis of the monodendron were conducted following the protocol described in Example 1), using Fmoc-Gly-Orn(Fmoc)-Gly-Gly-OH and the same solvents and reagents. Yield: 480 mg. After cleavage with TFA/water 95/5 v/v, the crude was purified by SEC on Sephadex G50, using 50% acetic acid as eluant. The fractions containing the target product were diluted with water and lyophilized three times. MS: 41,980 Da (Theor.: 41,972 Da).

2) Synthesis of N{CH_2-CH_2-N(-CO-)-CO-CH-S-CH_2-CH(COOH)-NH-CO(CH_2)$_3$-O



-CH(CH_3)-NH-Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn(Ac)-Gly-Ac]$_2$]$_2$]$_2$]$_2$]$_2$]$_2$]$_2$}$_3$

3.86 mg of Tris(2-maleimidoethyl)amine (Pierce) (0.01 mmole) and 4.0 g of



HOOC-CH(CH_2-SH)-NH-CO(CH_2)$_3$-O-...-CH(CH_3)-NH-Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-

Orn-Gly[Gly-Gly-Orn(Ac)-Gly-Ac]$_2$]$_2$]$_2$]$_2$]$_2$]$_2$ (0.1 mmole) were dissolved in 5 ml of DMF/water 10/90 v/v at room temperature under agitation and left to react for 3 h at an apparent pH of 7.0. 10 g of thiol-Sepharose 4B resin, preactivated with 2,2'-dipyridyldisulfide, were then added to the solution to sequester the unreacted monodendron by thiol-disulfide exchange in 7 ml of PBS buffer (pH 7.3). After elimination of the resin, the solution was evaporated, diluted with water and lyophilized. The crude was subsequently purified by SEC on Sephadex G50, using 50% acetic acid as eluant. The fraction containing the target product were diluted with water and lyophilized. Yield: 751 mg. MS: 126,309 Da (Theor.: 126,299 Da).

EXAMPLE 4

This example shows the stability of the polypeptide dendrimers described in Examples 1-3 to enzymatic hydrolysis in vitro.

The degradation in vitro was studied against Leucine-aminopeptidase VI (E.C. 3.4.11.1), isolated from pig kidneys, whose activity has been previously checked with leucine-4-nitroanilide. Dendrimer concentration: 1.10^{-3} M in 50 mM Tris.HCl buffer, pH 8.5, containing 5mM $MgCl_2$. Enzyme concentration: 3 U/ml. The experiments were performed at 37°C in an oscillating bath. Samples (100 µl each), withdrawn at fixed time intervals, were blocked with 10% TFA and centrifuged (10.000 x g , 5 min) before HPLC measurements that were performed on a Waters mod. 660 apparatus equipped with a Lichrosorb RP 18 (10µm) column. Detection was by a Jasco Uvidec-100-II detector. Eluant A was 0.1% TFA in water; and eluant B was 0.1% TFA in acetonitrile; gradient: from 0% B to 21% B in 23 min.

The degradation in heparinated human plasma was studied using dendrimer concentrations of ca. 1.0 nmole/ml plasma at 37°C, as described above. The extent of degradation with time was obtained by comparing the area of the HPLC signals appearing at a given time to that registered initially. The half-life of the generation 4 dendrimer with free amino terminals is ca. 12 h against Leucine-aminopeptidase VI and ca. 8 h in human plasma. The acetylated generation 4 and 7 dendrimers resulted less labile either to enzymatic degradation by Leucine-aminopeptidase VI (half-life, 23 h) or in human plasma (half-life, 16 h).

EXAMPLE 5

This example illustrates the loading by diffusion of the Enkephalinase inhibitor L-Trp-L-Ala in a generation 6 polypeptide dendrimer prepared as in Example 1) and its release with time.

30 mg of a generation 6 polypeptide dendrimer with free amino terminals, prepared as in Example 1), were added to 2 ml of an aqueous solution of 8 mg of L-Trp-L-Ala and after 24 h the clear solution was precipitated with 15 ml of ethanol under stirring. The precipitate was centrifuged, washed with anhydrous ethanol and dried under vacuum over P_2O_5. Yield: 29 mg. 10 mg of the isolated product were then dissolved in 10 ml of water and the solution injected into a 3-15 ml "Slide-A-Lyzer Dialyzer Cassette" (Pierce) ("cut-off", 10,000 Da). The dialysis was run against 100 ml of water for 48 h under slow stirring. The absorbance at 280 nm of 200 µl solution aliquots, diluted with water to a final volume of 1 ml, was determined every 30 min. Increasing absorbance values observed during ca. 12 h of dialysis indicated a gradual release with time of the dipeptide by slow diffusion from the dendrimeric carrier. A_{280} of the solution outside the dialysis cassette resulted slightly lower (-6%) than that of a reference solution prepared by dissolving 10 mg of dipeptide in 110 ml of water.

EXAMPLE 6

This example illustrates: a) the entrapment of heparin into a generation 7 polypeptide dendrimer containing photolabile bonds during condensation of the generation 7 monodendron to a trifunctional core carried out in the presence of heparin and b) the release of heparin by photolysis of the loaded dendrimer.

1). 1.12 g of sodium heparinate (obtained by depolimerization of ovine heparin, MW ca. 2,500 Da; activity, ca.180 IU/mg.) were added to the reagents used in Example 3.2 for the synthesis of the generation 7 dendrimer, at an apparent pH of 7.0. The monodendron condensation was protracted for 3 h at room temperature. After elimination of the monodendron in excess with thiol-Sepharose 4B resin, preactivated with 2,2'-dipyridyldisulfide, and filtration of the resin, the resulting clear solution was directly loaded on a. Sephadex G-75 column. The dendrimer was eluted with water at a flow of 0.5 ml/min to separate it from the excess of heparin. Yield: 1.04 g.

2). 750 mg of "loaded" dendrimer were dissolved in 6 ml of water and irradiated at

360 nm for 600 min in a quartz vial. Then, 1 ml of the irradiated solution was injected intravenously to each of six male rats (ca. 600 g body weight) deprived of food for 12 h before the beginning of the experiment (Rats 3-8). The same procedure was repeated using 1 ml of a non-irradiated solution of 750 mg of "loaded" dendrimer dissolved in 6 ml of water for six male rats of similar weight (Rats 9-14). Rat 1 is not injected at all, while rat 2 receives an intravenous injection of 250 mg of heparin dissolved in 1 ml of water. The anticoagulant effect of heparin i.e. the time needed to form a fibrin clot for serum samples taken from the vein of the tail, was ascertained by the APTT (Activated-Partial Thromboplastin Test) test. The results are reported below

		Coagulation time (seconds)					
Rat	**Treatment**	**t=0**	**1 h**	**2 h**	**3 h**	**4 h**	**24 h**
1		25	26	25	-	-	-
2	Heparin (iv)	27	54	>300	-	-	-
3	irradiated dendrimer	25	53	>300	-	-	-
4	id	26	57	>300	-	-	-
5	id	25	54	>300	-	-	-
6	id	24	56	>300	-	-	-
7	id	26	55	>300	-	-	-
8	id	27	54	>300	-	-	-
9	non-irradiated dendrimer	27	36	85	130	260	28
10	id	26	39	91	149	252	26
11	id	25	34	90	141	257	28
12	id	26	37	89	153	260	29
13	id	25	40	94	160	268	28
14	id	26	38	89	156	259	25

Within two hours, rats 3-8 showed coagulation times close to those of rat 2, treated with heparin only. Rats 9-14, treated with the non-irradiated dendrimer, showed an increase of coagulation times during four hours. At the first hour, the coagulation times are slightly less than that observed for rat 2 after two hours from heparin injection. The coagulation times for rats 9-14 becomes normal after 24 h. All together, the above results indicate that: a) low MW heparin is entrapped inside

the dendrimeric carrier; b) photolysis of the photolabile residue incorporated in the dendrimer backbone determines the release of heparin from the carrier and c) the non-irradiated dendrimer gradually releases heparin in parallel with the slow enzymatic demolition of its structure in blood.

EXAMPLE 7

This example reports: a) the absence of immunogenicity in mice of the generation 4 dendrimer obtained as described in Example 2) and b) its adjuvanticity when some of the NH_2 terminals are covalently linked to the octapeptide antigen Asn-Ala-Asn-Pro-Asn-Ala-Asn-Pro (a short segment of the immunodominant epitope of the Plasmodium falciparum Circumsporozoite Protein).

1) Immunogenicity of N{CH_2-CH_2-NH-CO-CH(CH_2-phenyl)-NH-Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly[Gly-Gly-Orn-Gly-H]$_2$]$_2$]$_2$}$_3$

50 μg of acetylated dendrimer were dissolved in 50 μl of Freund Complete Adjuvant and injected to 5 groups of C57/8L/6 mice (7-10 mice per group) at the base of the tail. After 3 weeks, 25 μg of dendrimer, emulsionated in 25 μl of Freund Incomplete Adjuvant, were injected to mice following the same procedure. After 10 days, a blood sample was taken from each mice by puncturing the retro-orbital plexus. Plasma samples were evaluated for the presence of anti-dendrimer antibodies by ELISA. Briefly, microtitre 96-well plates (Maxisorp F 96, Nunc, Denmark) were coated overnight in a humid chamber at 4°C with 100 μl of a solution containing 1 μg/ml of acetylated dendrimer in PBS at pH 7.2. Plates were then saturated with PBS and 5% non-fat dry milk for 2 h at room temperature. After three washings (phosphate buffer, pH 7.4 and 0.05% Tween-20), sera that were serially diluted in PBS, 2.5% non-fat dry milk and 0.05% Tween 20 were added to the plates for 1 h at room temperature. After washings, rabbit anti-mice IgG-specific polyvalent immunoglobulins conjugated to alkaline phosphatase, diluted in PBS, 2.5% non-fat dry milk and 0.05% Tween 20 were added for 1 h. Plates were washed and the presence of enzyme evidenced with p-nitrophenylphosphate substrate. Absorbance at 405 nm was measured with a Dynatech 25000 ELISA reader. Antidendrimer antibodies were not detected. To avoid the risk of removal of the dendrimer from the wells during reiterated washings, the experiments were repeated after conjugation (DCI/ HOBt as

coupling reagents, room temperature, 24 h) of the non-acetylated dendrimer to polyethylene pins, γ-irradiated in a 6% v/v aqueous solution of acrylic acid (M. Geysen et al., Proc. Natl. Acad. Sci., USA, 1984, 81, 3998-4002). The antidendrimer antibodies were detected by dipping the polyethylene pins into the wells of the microtitration plate, operating as described before. No antidendrimer antibodies were again detected in mice sera.

2) Conjugation of Asn-Ala-Asn-Pro-Asn-Ala-Asn-Pro to $N\{CH_2\text{-}CH_2\text{-}NH\text{-}CO\text{-}CH(CH_2\text{-phenyl})\text{-}NH\text{-}Gly\text{-}Gly\text{-}Orn\text{-}Gly[Gly\text{-}Gly\text{-}Orn\text{-}Gly[Gly\text{-}Gly\text{-}Orn\text{-}Gly[Gly\text{-}Gly\text{-}Orn\text{-}Gly\text{-}H]_2]_2]_2\}_3$

400.6 mg of $N\{CH_2\text{-}CH_2\text{-}NH\text{-}CO\text{-}CH(CH_2\text{-phenyl})\text{-}NH\text{-}Gly\text{-}Gly\text{-}Orn\text{-}Gly[Gly\text{-}Gly\text{-}Orn\text{-}Gly[Gly\text{-}Gly\text{-}Orn\text{-}Gly[Gly\text{-}Gly\text{-}Orn\text{-}Gly\text{-}H]_2]_2]_2\}_3$ (1.8 mmole) were dissolved in 10 ml of DMF together with 636 mg of Fmoc-Asn-Ala-Asn-Pro-OH (1.0 mmole), 192 mg of WSC·HCl (1.0 mmole), 154 mg of HOBt (1.0 mmole) and 460 μl of TEA. The solution, brought at basic pH with TEA, was stirred for 10 h at room temperature and then treated with 218.1 mg of $(Boc)_2O$ (1.0 mmole) after addition of 500 μl of TEA. The mixture was kept under agitation for 10 h, treated with 5 ml of piperidine, stirred for 2 h and finally precipitated by adding 100 ml of ethyl ether. The product was dissolved in 10 ml of water and purified by SEC on Sephadex G-50 using 50% acetic acid as eluant. The fractions containing the target product were recovered by lyophilization after dilution with water. 400 mg of the solid were again dissolved in 10 ml of DMF and the coupling of Fmoc-Asn-Ala-Asn-Pro-OH to the dendrimer was repeated once more. After addition of 5 ml of 20% piperidine in DMF, and stirring for 3h at room temperature, 100 ml of ethyl ether were added to precipitate the product. Yield: 305 mg. The compound was again dissolved in 5 ml of TFA/water 95/5 v/v and, after one hour at room temperature, 100 ml of ethyl ether were added to precipitate a white powdery solid. Following drying over P_2O_5 in vacuum, the crude was purified by SEC on Sephadex G-50 Superfine, using 50% acetic acid as the eluant. Yield: 280 mg.

3) Assessment of the adjuvant properties of $N\{CH_2\text{-}CH_2\text{-}NH\text{-}CO\text{-}CH(CH_2\text{-phenyl})\text{-}NH\text{-}Gly\text{-}Gly\text{-}Orn\text{-}Gly[Gly\text{-}Gly\text{-}Orn\text{-}Gly[Gly\text{-}Gly\text{-}Orn\text{-}Gly[Gly\text{-}Gly\text{-}Orn(\text{-}Pro\text{-}$

Asn-Ala-Asn-Pro-Asn-Ala-Asn)-Gly-Pro-Asn-Ala-Asn-Pro-Asn-Ala-Asn]2]2]2}3.

Each component of five groups of BALB/c female mice, 7-10 mice per group, (OLAC, Bicester, Oxon, UK) was injected with 500 μg of antigen-dendrimer conjugate dissolved in 50 ml of water as described before. In parallel, the same number of C57/8L/6 mice were injected with 50 μg of Asn-Ala-Asn-Pro-Asn-Ala-Asn-Pro dissolved in 50 μl of water. After three weeks, 25 and 250 μg of the same products were injected again to the two groups of mice. 10 days after, a sample of blood was taken from each mice as described before. The sera were tested by an ELISA test employing $(Asn\text{-}Ala\text{-}Asn\text{-}Pro)_{40}$ as the antigen. (G. Del Giudice et al., J. Clin. Microbiol, 1997, 25, 91-96). The antigen-dendrimer conjugate shows higher anti-Asn-Ala-Asn-Pro antibody titers (as the logarithmic geometric mean of antibody titers ± S.E.M.) at week 45 (4.10±0.01) as compared to Asn-Ala-Asn-Pro-Asn-Ala-Asn-Pro antigen (2.81±08).

Taking into account all the above results, the polypeptide dendrimers of the present invention, obtained by chemical synthesis, satisfy the foreseen objectives. In particular, unimolecular polypeptide dendrimers can be obtained with the processes of synthesis described and, furthermore, the practicality of dendrimer loading and of controlled release of guest molecules in vivo by enzymatic hydrolysis and through the application of ultraviolet irradiation has been demonstrated. Applications of the unimolecular carrier polypeptide dendrimers/guest molecules system in composition with pharmaceutically acceptable excipients in the medical field are widespread and potentially of extreme importance namely chemotherapy of cancer, anticoagulant and clot-dissolving drug therapy, antiviral therapy, vaccines, controlled release of hormones and related bioactive substances. For medical diagnosis, the controlled methods of synthesis described above give the possibility to prepare metal chelates of dendrimeric carriers with precisely defined molecular weights, so that the drawbacks due to the presence of imperfect carrier structures are avoided. Applications to medical diagnosis and therapy are no meant to be restricted to those implementations described, as many other possibilities will be clear to one skilled in the medical arts.

CLAIMS

1. A polypeptide dendrimer having: i) a multifunctional core moiety; ii) an exterior of closely spaced groups constituting the terminals of branched polypeptide chains (monodendrons) radially attached to the core that, in turn, form iii) interior layers (generations) of short peptide branching units (propagators) with characteristic hollows and channels where each propagator contains a trifunctional aminoacid whose asymmetric carbon (the propagator branching point) is connected to two equal-length arms bearing identical terminal reactive groups and to a third arm (the propagator stem) bearing an activatable functional group,
represented by formula (I):

$K(-L)_p-M$ (I) wherein

K is a multifunctional core moiety,
L is a polypeptide monodendron,
p is the number of polypeptide monodendrons irradiating from the core moiety and M represents the outermost ramifications of the dendrimer;

2. A polypeptide dendrimer of claim 1 where said K is represented by formula (II):

$X-(CH_2)_n-X^1$ (II)

wherein $X=X^1$ or $X\neq X^1$, and X, X^1 are NH or CO or S;

3. A polypeptide dendrimer of claim 1 where said K is represented by formula (III):

$Y[-(CH_2)_n-Z]_i$ (III)

wherein Y=C or Y=N; Z is NH or S or Cl or Br or I or a maleimide residue, n=1-6 and i=3,4;

4. A polypeptide dendrimer of claim 1 where said K is represented by formula (IV):

$$X-CH(R)-CO[-NH-CH(R)-CO]_n-NH-CH(R)-COOR^1 \quad (IV)$$

wherein R is $(CH_2)_m-X^1$, m=1-5, R^1 is methyl or ethyl or butyl or isopropyl, $X=X^1$ or $X\neq X^1$, and X, X^1 are NH or CO or S and n=1-6;

5. A polypeptide dendrimer of claim 1 where said L is the single monodendron whose propagators are represented by formula (V):

$$-CO-CH(R^2)-(CH_2)_n-NR^3- \quad (V)$$

wherein R^2=H or the side-chain of natural or synthetic aminoacids, and their derivatives; R^3=H or a linear hydrocarbon radical optionally substituted with OH or SH or Cl or Br; R^2-$CH(CH_2)_n$-NR^3 is a 5 or 6 atoms ring, and n=0-6;

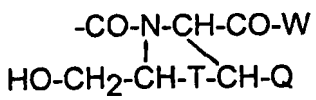
6. A polypeptide dendrimer of claim 1 where said L is the single monodendron whose propagators are represented by formula (VI):

$$-CO-CH(R^2)-CO-N(R^3)-(CH_2)_m-N(R^3) \qquad (VI)$$

wherein R^2 and R^3 have the meaning seen in claim 5 and m=1-6;

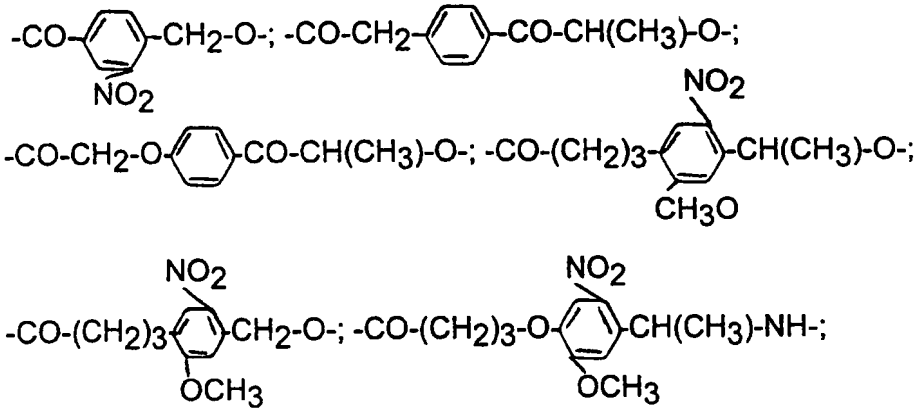
7. A polypeptide dendrimer of claim 1 where said L is the single monodendron whose propagators are represented by one of the residues:

$-CO-CH_2-NH-NH-$; or $-CO-CH(R^2)-O-$; or $-CO-CH_2-O-N=CH-CO-$; or $-CO-CH(R^2)-(CH_2)_n-S-CH_2-CO-W$; or $-CO-NH-CH(CH_2-SH)-CO-W$ or



wherein W=$-N(R^3)-(CH_2)_m-NR^3$, Q=H or $-CH_3$; T is O or S whereas R^2, R^3 and m have the meaning seen in claim 5;

8. A polypeptide dendrimer of claim 1 where said L is the single monodendron whose propagators are represented by one of the residues:



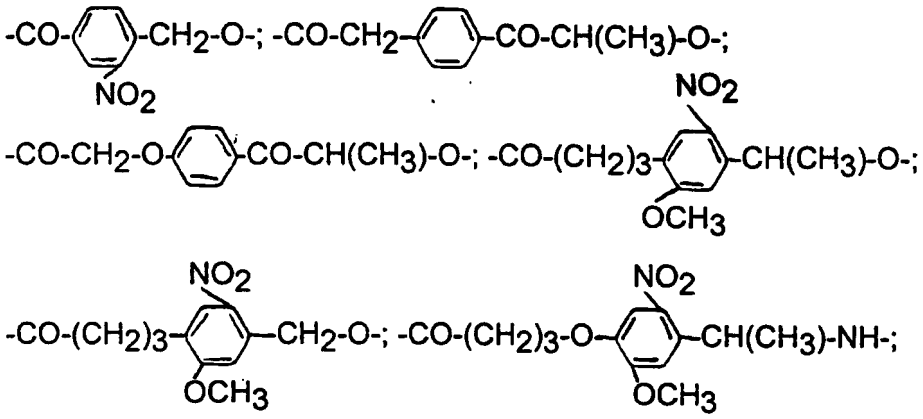
9. A polypeptide dendrimer of claim 1 where said p is 1 or 2 or 3 or 4;

10. A polypeptide dendrimer of claim 1 where said M is the residue represented by formula (VII):

$$-Aq-B(Ar)-C-Ar[Aq-B(Ar)-C-Ar[Aq-B(Ar-D)-C-Ar-D]_2]_2 \qquad (VII)$$

wherein A=$-CO-CH(R^2)-(CH_2)n-NR^3$, R^3 and n have the meaning seen in claim 5, q=1-6, r=1-4 and R^2, in addition to the meaning seen in claim 5, is a natural or

synthetic trifunctional aminoacid; B is -CO-CH[-(CH_2)n-X^1]-X, with X=X^1 or X≠X^1; X and X^1 are NH or CO or S; n=1-5; C=A or C=-CO(CH_2)n-NH- or -(CH_2)n-S- with n=1-6 or C is one of the residues:



D is a residue represented by formulae (VIII)-(XI):

-Aq-B(Ar-E)-C-Aq-E (VIII)

-Aq-B(Ar)-C-Aq[Aq-B(Ar-E)-C-Aq-E]$_2$ (IX)

-Aq-B(Ar)-C-Aq[Aq-B(Ar)-C-Aq-[Aq-B(Ar-E)-C-Aq-E]$_2$]$_2$ (X)

-Aq-B(Ar)-C-Aq[Aq-B(Ar)-C-Aq-[Aq-B(Ar)-C-Aq[Aq-B(Ar-E)-C-Aq-E]$_2$]$_2$]$_2$ (XI)

wherein A, B, C, q ed r have the meaning seen above , and E is represented by formulae (XII) and (XIII):

-Aq-B(Ar-P)-C-Aq-P^1 (XII)

-Aq-B(Ar)-C-Aq[-Aq-B(Ar-P)-C-Aq-P^1]$_2$ (XIII)

wherein A, B, C, q and r have the meaning seen above, P=P^1 or P≠P^1, P and P^1 being H or a linear hydrocarbon radical optionally substituted with one or more linear or branched alkyl groups, acyl, aminoacid, peptide, nucleotide, oligonucleotide, saccharide, oligosaccharide, protein, monoclonal antibody, polyethyleneglycol containing 10-400 -CH_2-CH_2-O- repeats, lipid, enzyme, metal ligand or their synthetic analogues and derivatives;

11. A polypeptide dendrimer of claims 1-10 wherein the two-dimensional molecular diameter of the dendrimers is in the range from about 10 to 100 nm.

12. The dendrimer $_2$($_2$($_2$(H-Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly-HN-CH_2-CH_2-NH-Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly-H)$_2$)$_2$)$_2$.

13. The dendrimer $_2$($_2$($_2$($_2$(H-Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-

Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly-HN-CH_2-CH_2-NH-Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly-H)$_2$)$_2$)$_2$)$_2$.

14. The dendrimer $_2$(2(2(2(2(H-Gly-Orn-Gly-Gly]Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly-HN-CH_2-CH_2-NH-Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly-H)$_2$)$_2$)$_2$)$_2$)$_2$.

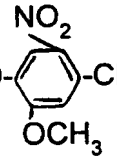
15. The dendrimer $_2$(2(2(2(2(2(H-Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly-HN-CH_2-CH_2-NH-Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly-H)$_2$)$_2$)$_2$)$_2$)$_2$)$_2$.

16. The dendrimer $_2$(2(2(2(2(2(2(H-Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly)Gly-Orn-Gly-Gly-HN-CH_2-CH_2-NH-Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly(Gly-Gly-Orn-Gly-H)$_2$)$_2$)$_2$)$_2$)$_2$)$_2$.

17. The dendrimer N{-CH_2-CH_2-NH-CO-CH(-CH_2-phenyl)-NH-Gly-Gly-Gly-Orn-Gly[Gly-Gly-Gly-Orn-Gly[Gly-Gly-Gly-Orn-Gly[Gly-Gly-Gly-Orn-Gly-H]$_2$]$_2$]$_2$}$_3$.

18. The dendrimer N{-CH_2-CH_2-NH-CO-CH(-CH_2-phenyl)-NH-Gly-Gly-Gly-Orn-Gly[Gly-Gly-Gly-Orn-Gly[Gly-Gly-Gly-Orn-Gly[Gly-Gly-Gly-Orn-Gly[Gly-Gly-Gly-Orn-Gly-H]$_2$]$_2$]$_2$]$_2$}$_3$.

19. The dendrimer N{-CH_2-CH_2-N(-CO-CH-S-CH_2-CH(COOH)-NH-



-$CO(CH_2)_3$-O-

-$CH(CH_3)$-NH-Gly-Gly-Gly-Orn-Gly[Gly-Gly-Gly-Orn-Gly[Gly-Gly-Gly-Orn-Gly[Gly-Gly-Gly-Orn-Gly[Gly-Gly-Gly-Orn-Gly[Gly-Gly-Gly-Orn-Gly-H]$_2$]$_2$]$_2$]$_2$]$_2$]$_2$}$_3$.

20. The polypeptide dendrimers of claims 12-19 wherein the NH_2 terminals are acetylated.

21. A polypeptide dendrimer of claim 1 wherein at least one bioactive or marker molecule is covalently linked to the surface of the same.

22. A polypeptide dendrimer of claim 21 where the bioactive molecule is selected in the group comprising an aminoacid, a peptide, a protein, a nucleotide, an oligonucleotide, a lipid, a saccharide, an oligosaccharide, and a small organic molecule and their synthetic analogues and derivatives.

23. A polypeptide dendrimer of claim 21 where the bioactive molecule is selected in the group comprising drugs, cellular receptor ligands, bacterial, viral and parasite antigens and gene-therapy compounds.

24. A polypeptide dendrimer of claim 21 where the marker molecule is a diagnostic imaging contrast agent.

25. A polypeptide dendrimer of claim 1 where the bioactive molecule is entrapped in the same.

26. A polypeptide dendrimer of claim 25 where the bioactive molecule is selected in the group comprising an aminoacid, a peptide, a protein, a nucleotide, an oligonucleotide, a lipid, a saccharide, an oligosaccharide, and a small organic molecule and their synthetic analogues and derivatives.

27. A polypeptide dendrimer of claim 25 where the bioactive molecule is selected in the group comprising drugs, cellular receptor ligands, bacterial, viral and parasite antigens and gene-therapy compounds.

28. A polypeptide dendrimer of claim 27 where the bioactive molecules are anticancer drugs.

29. A polypeptide dendrimer of claim 27 where the bioactive molecules are antibiotics.

30. A polypeptide dendrimer of claim 27 where the bioactive molecules are antiviral substances.

31. A process for production of the polypeptide dendrimers of claim 1 characterized by the following steps:

i) synthesis of core moieties with at least two reactive functional groups;

ii) divergent synthesis on solid-phase of polypeptide monodendrons with temporarily or permanently protected terminals;

iii) covalent condensation of polypeptide monodendrons to core moieties;

32. A process for production of polypeptide dendrimers of claim 1 characterized by the following steps:

i) synthesis of core moieties with at least two reactive functional groups;

ii) covalent condensation to the core moieties of polypeptide monodendrons of generation 1-3 with temporarily protected terminals to obtain the corresponding protected dendrimers;

iii) after protecting groups removal, repeated condensations of polypeptide monodendrons to the dendrimer reactive terminals to obtain the desired final dendrimers.

33. A process for entrapping into the polypeptide dendrimers of claim 1 bioactive substances and drugs with molecular weights lower than 1,000 Da, characterized by the following steps:

(a) adding suitable amounts of polypeptide dendrimers to a concentrated or saturated solution of said molecules and

(b) precipitating the loaded polypeptide dendrimer after 24 h incubation at room temperature in a large volume of a precipitant.

34. A process for entrapping into the polypeptide dendrimers of claim 1 bioactive substances and drugs with molecular weights higher than 1,000 Da, characterized by the selective chemical ligation of polypeptide monodendrons, in aqueous buffers, to the core moieties in the presence of said molecules.

35. A process for the selective chemical ligation of bioactive substances and drugs to the internal functional groups of the polypeptide dendrimers of claim 1, in aqueous buffers, after loading the dendrimer carrier by diffusion.

36. Use of polypeptide dendrimers of claim 1 as unimolecular carriers of bioactive molecules wherein at least one bioactive or marker molecule is covalently linked to the surface of the same.

37. Use of polypeptide dendrimers according to claim 36 where the bioactive molecule is selected in the group comprising an aminoacid, a peptide, a protein, a nucleotide, an oligonucleotide, a lipid, a saccharide, an oligosaccharide, and a small organic molecule and their synthetic analogues and derivatives.

38. Use of polypeptide dendrimers according to claim 36 where the bioactive molecule is selected in the group comprising drugs, cellular receptor ligands, bacterial, viral and parasite antigens and gene-therapy compounds.

39. Use of polypeptide dendrimers according to claim 36 where the marker

molecule is a diagnostic imaging contrast agent.

40. Use of polypeptide dendrimers of claim 1 as unimolecular carriers of bioactive molecules wherein the bioactive molecule is entrapped into the same.

41. Use of polypeptide dendrimers according to claim 40 where the bioactive molecule is selected in the group comprising an aminoacid, a peptide, a protein, a nucleotide, an oligonucleotide, a lipid, a saccharide, an oligosaccharide, and a small organic molecule and their synthetic analogues and derivatives.

42. Use of polypeptide dendrimers according to claim 40 where the bioactive molecule is selected in the group comprising drugs, cellular receptor ligands, bacterial, viral and parasite antigens and gene-therapy compounds.

43. Use of polypeptide dendrimers according to claim 40 where the bioactive molecules are anticancer drugs.

44. Use of polypeptide dendrimers according to claim 40 where the bioactive molecules are antibiotics.

45. Use of polypeptide dendrimers according to claim 40 where the bioactive molecules are antiviral substances.

46. Compositions with pharmaceutically acceptable excipients wherein the polypeptide dendrimers of claim 1 are the unimolecular carriers of bioactive or marker molecules covalently linked at the surface of the same.

47. Compositions with pharmaceutically acceptable excipients wherein the polypeptide dendrimers of claim 1 are the unimolecular carriers of bioactive molecules entrapped into the same.

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(54) Title: POLYPEPTIDE DENDRIMERS AS UNIMOLECULAR CARRIERS OF DIAGNOSTIC IMAGING CONTRAST AGENTS, BIOACTIVE SUBSTANCES AND DRUGS

(57) Abstract: The invention describes new polypeptide dendrimers and processes for the synthesis of the same. The polypeptide dendrimers of the invention have a structure which consists of a multifunctional core moiety from which highly branched polypeptide chains, formed by short peptide branching units, extend radially outwards. The outermost branches surround a lower density space with hollows and channels into which bioactive substances employed in diagnosis and therapy can be entrapped or covalently linked. For these properties the said polypeptide dendrimers are particularly useful in a number of areas in biology and medicine as carriers for the delivery of bioactive substances, including drugs, or as carriers of bacterial, viral and parasite antigens, gene-therapy compounds and diagnostic imaging contrast agents.

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/EP 00/07022

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/00 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 00540 A (WEBBER ROBERT) 5 January 1995 (1995-01-05)	1-11, 20-24, 31,32, 36-39,46
Y	The whole document; see esp. page 9 and Fig.1	1-11, 20-47
X	HUANG E.A.: "Lipophilic multiple antigen peptide system for peptide immungen and synthetic vaccin" MOL.IMMUNOL., vol. 31, no. 15, 1994, pages 1191-1199, XP002160052 See especially Fig.3a and 5	1,2,4,9, 20-23, 31,32, 36-38,46

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
12 February 2001	22/02/2001

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INTERNATIONAL SEARCH REPORT

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